

Current State in The Diagnosis of Blood Parasite *Babesia sp*

Tatik Suteky

Jurusan Peternakan Fakultas Pertanian Universitas Bengkulu
Jalan Raya Kandang Limun Bengkulu Telp. (0736) 21170 Pst 219

ABSTRAK

Babesiosis merupakan penyakit parasit yang disebabkan oleh *Babesia sp* tidak hanya menyerang berbagai spesies hewan tetapi juga pada manusia. Kasus babesiosis pada orang yang disebabkan oleh *Babesia microti* telah dilaporkan diberbagai negara. Diagnosis untuk *Babesia* biasanya dilakukan secara mikroskopis dengan preparat apus darah, namun dengan metode ini sering tidak menunjukkan hasil akurat sehingga metode lain perlu dikembangkan. Metode seperti sub inokulasi pada hewan percobaan dan Quantitative Buffy Coat (QBC) System, dianggap kurang praktis, tes serologi seperti dengan IFAT dan ELISA yang telah dikembangkan dan mudah untuk di standarisasi menunjukkan spesifitas dan sensitivitas yang tinggi. Penggunaan Immunobloting untuk mendiagnosis babesiosis terhadap 24 pasien menunjukkan spesifitas 99% dan prediksi positif serta prediksi negatif sebesar 96 % dan 99 %.

Kata Kunci : Babesiosis, diagnosis, IFAT, ELISA dan Immunobloting

ABSTRACT

Babesiosis caused by the protozoan parasite and is frequently fatal not only in number of animal but also in human. Prompt and accurate diagnosis is essential to effective case management because the condition tends to be so local and potentially so severe. Although conclusive diagnosis of this disease generally depends upon microscopic examination of thin blood smears, the pathogenic *Babesia* frequently are overlooked because parasitemia tends to be sparse, often infecting fewer than 1% of erythrocytes early in the course of the illness. Quantitative Buffy Coat (QBC) and animal sub inoculation were not really reliable, serological test such as IFAT and ELISA is sensitive and specific may be easier to standardize and to perform also a very useful technique for mass screening. In addition, the sensitivity immunoblot test in 24 patients caused by *B. microti* was 96%, while specificity was 99% and predictive positivity and predictive negativity were 96 and 99%, respectively

Key words : Babesiosis, diagnosis, IFAT, ELISA and Immunoblot test

INTRODUCTION

Babesiosis is a malaria-like disease, caused by the protozoan parasite and is frequently fatal in a number of animal species. Cases of human babesiosis caused by the rodent parasite *Babesia microti* have been reported in the USA and Mexico by several workers (Krause *et al.*,1991). Recent epidemiological studies have been conducted on *Babesia microti* because of its prevalence in humans in the northeast of North America. This malaria-like infection that may be life-threatening and is endemic in parts of the northeastern and north central United States

Specific diagnosis of babesiosis can be made by microscopic identification of the organism in Giemsa stained thin blood smears, detection of specific antibody in acute and convalescent sera.

Thin Blood Smear

The advantage of thin blood films is the excellent demonstration of the morphological detail of *Babesia* parasites, although specialist training may be needed to differentiate species that can appear morphologically similar. Likewise, the ring form of *Babesia* seen in a stained blood film may be easily mistaken for

the early ring form of *Plasmodium* species. Indeed, for post mortem diagnosis of freshly dead animals from any organ such as spleen, liver, kidney, heart muscle and brain can be used. In decomposing animals, however, samples should only be taken from the spleen or brain (Bose *et al.*, 1995).

Thick Blood Film

A thick blood film permits examination of a larger volume of blood and consequently is much more sensitive than thin films. Thick blood films have been used to detect some species of *Babesia* which have a very low parasitaemia. However, the small size of *Babesia microti*, does not allow for their confident recognition within thick blood smears (Ash and Orihel, 1987; Persing *et al.*, 1992; Bose *et al.*, 1995).

Quantitative Buffy Coat (QBC) System

This system has been designed to detect *Plasmodium* species in human blood (Spielman *et al.*, 1988) and has also been employed to detect *Babesia microti* in human blood (Mattia *et al.*, 1993). The QBC method utilises acridine orange to stain parasites within specialised capillary tubes. Infected cells are pelleted by differential centrifugation and visualised using fluorescent epi-illumination. The predominant morphologic appearance of *Babesia microti* prepared by QBC method is a tiny bright chromatin dot surrounded by a small round or pyriform shaped-body of cytoplasm. This technique is reportedly eight to ten times more sensitive than the examination of Giemsa-stained blood film (Bose *et al.*, 1995). However, the current limitation of the QBC analysis is the cost of equipment (Mattia *et al.*, 1993).

Animal Sub-inoculation / Isotest

Subinoculation of carrier blood or blood drawn from wild animals into susceptible or splenectomised animals has been the method of choice to detect *Babesia microti* in wild animals (Etkind *et al.*, 1980) and in humans (Brandt *et al.*,

1977) as well as sub-patent infection in rodent malaria during pregnancy (Oduola *et al.*, 1982; Vinayak *et al.*, 1986). However, such a method requires several weeks for completion which is impractical for diagnostic purposes (Brandt *et al.*, 1977).

Indirect Fluorescent Antibody Technique (IFAT)

Fluorescent antibody methods have been used extensively for the serological diagnosis of animal and human babesiosis. Cox and Turner (1970a) employed specific anti-mouse total Ig, IgM and IgG in the IFAT to detect antibody in mice infected with *Babesia microti*. It was found that the Ig level rises rapidly with *Babesia microti* parasitaemia until the twelfth day of infection and continues until the parasites disappear. The IgM was almost parallel with Ig but declined as parasitaemia decreased. IgG levels, however, rose until the parasites disappeared from the blood. In other experiments, Cox and Turner (1970b) and Zivkovic *et al.* (1984) used the IFAT to determine antigenic similarities and differences between malaria and *Babesia* in mice. Cross reactions were observed between the two intra-erythrocytic protozoa.

Perez *et al.* (1977) employed the IFAT to measure humoral antibody responses in hamsters infected with *Babesia microti*. The titers increased during the first week after challenge, and then fluctuated. According to Krause *et al.* (1994), the IFA test is useful in the diagnosis of *Babesia microti* infection as well as a seroepidemiological tool to study the prevalence and distribution of *Babesia microti* infection in humans with a high sensitivity and specificity. Such a test could detect the development and persistence of antibody seven months after the onset of illness in cases of human babesiosis.

In addition, by employing IFAT, Krause *et al.* (1991) found that the percentage of reactive sera from Connecticut residents has increased in recent years. Nevertheless, the main disadvantages of this test are the cross-reactivity that occurred between *Babesia microti* and *Plasmodium* species. Therefore, the interpretation

of the results will depend on whether the patient could have been previously exposed to malaria. The range of test results in the four laboratories was 88%-96% sensitivity, 90%-100% specificity, 69%-100% positive predictive value, and 96%-99% negative predictive value. Interlaboratory and intralaboratory concordance ranged from 84% to 85% and 94% to 100%, respectively. This *B. microti* IFA procedure is a sensitive, specific, and reproducible method for diagnosing babesiosis and is suitable for use as a standard in laboratories testing human sera for *B. microti* antibody.

Western blot (WB) assay

Ryan *et al.* (1999) evaluated the usefulness of a *B. microti* Western blot (WB) assay (Immunetics, Cambridge, MA) in conjunction with an indirect immunofluorescence assay (IFA) for the diagnosis of babesiosis in patients suspected of having the disease. Thirty eight specimens from 29 patients were tested by both IFA and Western blot. Of the 29 patients tested, 15 (52%) had no detectable babesial antibody by IFA while 14 (48%) patients had one or more specimens positive for either IgM, IgG or both. All specimens which were positive by IFA were also positive by Babesia WB using the interpretive criteria specified. Thirteen of the 15 patients negative for babesial antibody by IFA were also negative by WB. Two specimens were negative by IFA and positive by WB. The data suggest that IFA and WB are both sensitive and specific and are useful for the laboratory diagnosis of *B. microti* infection. When two reactive bands were considered as definitive, immunoblot test sensitivity was 96%, while specificity was 99% and predictive positivity and predictive negativity were 96 and 99%, respectively. Our *B. microti* immunoblot procedure shows promise as a sensitive, specific, and reproducible assay for routine clinical diagnosis of acute babesiosis (Ryan *et al.*, 2001)

Enzyme-linked Immunosorbent Assay

In Japan, Akiba *et al.* (1991) tested the suitability of an enzyme-linked immunosorbent

assay (ELISA) for the detection of antibodies in BALB/c mice infected with *Babesia rodhaini*. Positive reactions in euthymic (nu/+) BALB/c mice were detected by day 7 but had declined by day 17 with the termination of parasitaemia. No antibody response was detected by an ELISA in athymic (nu/nu) BALB/c mice.

In addition, Cavacini *et al.* (1990) employed an ELISA using a biotinylated goat anti-mouse IgM and alkaline phosphatase-conjugate avidin system and found the level of IgM was only 1.44 µg/mL in B cell-deficient BALB/c mice infected with *Babesia microti*, level up to 5.5 mg/mL were recorded in infected immunocompetent BALB/c mice. According to Madruga *et al.* (2001) an indirect enzyme-linked immunosorbent assay (ELISA) using a crude antigen was evaluated for its performance to detect *Babesia bigemina* antibodies. The sensitivity and specificity were 98.0% and 99.0%, respectively

CONCLUSION

Rapid and accurate diagnosis is important to cope case management of Babesiosis. Microscopic examination of thin or thick blood smears, is commonly used, however these methods frequently are overlooked because parasitemia tends to fewer than 1%. Serological tests such as IFAT, ELISA and Western blot (WB) assay

shows promise as a sensitive, specific, and reproducible assay for routine clinical diagnosis of acute babesiosis.

REFERENCES

- Akiba M, Saeki H, Ishii T, Yamamoto S and K. Ueda 1991. Immunological change in *Babesia rodhaini* infected BALB/c mice after treatment with anti-babesial drug diaminazene diacetate. *Journal of Veterinary Medical Science* 53:371-377.
- Ash LR and T.C. Orihel 1987. **Parasites: A Guide to Laboratory Procedures and Identification**. ASCP Press, Chicago. pp.328.
- Brandt F, Healy G.R. and M. Welch 1977. Human babesiosis: the isolation of *Babesia microti* in golden hamsters. *Journal of Parasitology* 63:934-937.

- Bose R, Jorgensen W.K., Dalglish R.J., Friedhoff K.T. and A.J. .De Vos 1995. Current state and future trends in the diagnosis of babesiosis. *Veterinary Parasitology* 57:61-74.
- Bose R and A.G .Lacson 1995. Embryo-associated immunosuppressor factor is produced at the maternal-fetal interface in human pregnancy. *American Journal of Reproductive Immunology* 33:373-380.
- Cavacini L.A, Parker L.A and W.P. Weidanz 1990. Resolution of acute malarial infections by T cell-dependent non-antibody-mediated mechanism of immunity. *Infection and Immunity* 58:2946-2950.
- Cox F.E.G and S.A .Turner 1970a. Antibody levels in mice infected with *Babesia microti* . *Annals of Tropical Medicine and Parasitology* 64:167-173.
- Cox F.E.G and S.A. .Turner 1970b. Antigenic relationship between the malaria parasites and piroplasm in mice as determined the fluorescent-antibody technique. *Bulletin of World Health Organisation* 43:337-340.
- Etkind P, Piesman J, Ruebush T.K., Spielman A and D.D. Juranek 1980. Methods for detecting *Babesia microti* infection in wild rodents. *Journal of Parasitology* 66:107-110.
- Krause G, Telford III, S.R, Ryan R, Hurta A.B., Kwasnik I, Luger S, Niederman J, Gerber M and Spielman A 1991. Geographical and temporal distribution of babesiosis in Connecticut. *Journal Clinical Microbiology* 29:2779-2783.
- Krause PJ, Telford III S.R., Ryan R, Conrad P.A, Wilson M, Thomford J.W. and A.Spielman .1994. Diagnosis of babesiosis: evaluation of a serologic test or the detection of *Babesia microti* antibody. *Journal of Infectious Diseases* 169:923-926.
- Mattia AR, Waldron MA and L.S. Sierra 1993. Use of quantitative buffy coat system for detection of parasitemia in patients with babesiosis. *Journal of Clinical Microbiology* 31:2816-2818.
- Oduola A.M.J, Holbrook T.W., Galbraith R.M., Bank H and S.S.Spicer. 1982. Effect of malaria (*Plasmodium berghei*) on the maternal-foetal relationship in mice. *Journal of Protozoology* 29:77-81.
- Oduola A.M.J, Phillips J.H, Spicer S.S. and R.M. Galbraith 1986. *Plasmodium berghei*: histology, immunocytochemistry, and ultrastructure of the placenta in rodent malaria. *Experimental Parasitology* 62:181-193.
- Persing DH, Mathiesen D, Marshall WF, Telford SR, Spielman A, Thomford JW and PA Conrad. 1992. Detection of *Babesia microti* by polymerase chain reaction. *Journal of Clinical Microbiology* 30:2097-2103.
- Ryan R.W, Kowalski I, Oliwa N, Aslanzadeh J, P. Krause 1999. Use of Western Blot Assay for the Laboratory Diagnosis of *Babesia microti* Infection. *Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother*. Sep 26-29; 39: 239
- Spielman A, Perrone J.B., Teklehaimanot A, Balcha F, Wardlaw S.C and R.A .Levine 1988. Malaria diagnosis by direct observation of centrifuged samples of blood. *American Journal of Tropical Medicine and Hygiene* 39:337-342.
- Vinayak VK, Pathak G, Asnani PJ, Jain S and A.K. Malik 1986. Influence of malarial infection on the maternal-foetal relationship in pregnant mice. *Australian Journal of Experimental Biology and Medical Science* 64:223-237.
- Raymond R., P.J. Krause.,J R Adolf, K Freeman, A Spielman, R Lenz, and A. Levin. 1999. Diagnosis of Babesiosis Using an Immunoblot Serologic Test. *Clinical And Diagnostic Laboratory Immunology* 8:1177-1180
- Zivkovic D, Speksnijder J.E., Kuil H and W Seinen 1984. Immunity to *Babesia* in mice. II. Cross protection between various *Babesia* and *Plasmodium* species and its relevant to the nature of *Babesia* immunity. *Veterinary Immunology and Immunopathology* 22:359-368.